Norwegian Sea Herring Stock Discrimination phase I [NORDIS I]

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Summary

There is growing concern among fishermen about the migration of North Sea herring into the Norwegian Sea. The Pelagic Freezer-trawler Association therefore commissioned IMARES to develop a technique to monitor possible catches of North Sea herring in the Norwegian Sea. This technique will use morphometric (shape) differences in herring to distinguish between Norwegian Sea spawning herring and North Sea herring. Jaczon BV and Van der Zwan provided herring samples that were caught in the Norwegian Sea and were analysed within this project. Initial investigations showed that methods to use the morphometric analysis of the shape of a whole fish were not suitably advanced to be a cost effective mechanism for the discrimination of herring stocks, thus another approach was investigated.

DTU Aqua, Denmark, has developed a method in which the spawning origin of herring is determined by using morphometric shape discrimination of otoliths. Within this project we developed in house the laboratory methods that are needed for this technique. It involves taking a photograph of the complete ‘loose’, i.e. not embedded otolith followed by an Elliptical Fourier Analysis, which fits a closed curve around the outer contour of an otolith. This results in a list of parameters that can be run through a classification model. This model was set up with the use of a baseline that consisted of definite Norwegian spring, North Sea autumn and North Sea winter spawners. Thereafter the samples originating from the Norwegian Sea were analysed.

The results show that the model is able to distinguish Norwegian spring spawning herring from North Sea autumn or winter spawning herring. Based on the model 80% of the samples from the Norwegian Sea were classified as Norwegian spring spawning herring and 20% as other fish that would probably be North Sea autumn or winter spawning fish. A few samples contained fish with otolith shapes that were not covered by the baseline analysis. At present these samples are classified as either Norwegian spring spawners or North Sea autumn or winter spawners. However, it is possible that these samples come from a herring type that we have not included in the baseline. During this project we also briefly examined whether it is possible to apply the technique of morphometric shape discrimination on embedded otoliths. First results show that the embedding process can affect the outcome of the classification. Overall we can conclude that even though we still have to overcome some methodological problems we are confident that this research constitutes a first step towards developing a technique to monitor catches of herring from the Norwegian Sea for Norwegian Spring spawning or other herring.

Contents

1 Introduction

The North Sea herring (*Clupea harengus*) stock consists of a complex mixture of spawning components (Figure 1.1). Each spawning component has its own spawning grounds, migration routes and nursery areas (ICES, 2009). During the feeding season (summer) the subcomponents mix on the feeding grounds in the central North Sea where they feed mainly on plankton (ICES, 2009). The difference in spawning time and location results in the components experiencing different environmental conditions in, for example temperature and food availability, as they develop (Heath *et al.*, 1997). It appears that the growth rate of herring larvae is sensitive to such factors (Geffen, 2009). The growth rate is, in turn, directly linked to otolith shape and microstructure, with faster growth producing longer thinner crystals (Gauldie and Nelson, 1990).

There is growing concern among fishermen about the migration of North Sea herring into the Norwegian Sea. If North Sea herring is actually migrating and is caught outside the North Sea, the assessment of North Sea herring may be biased. The quality of the assessment may also be effected if there is a trend over time in the size of this migration. The Pelagic Freezer-trawler Association therefore commissioned IMARES to develop a technique to monitor possible catches of North Sea herring in the Norwegian Sea.

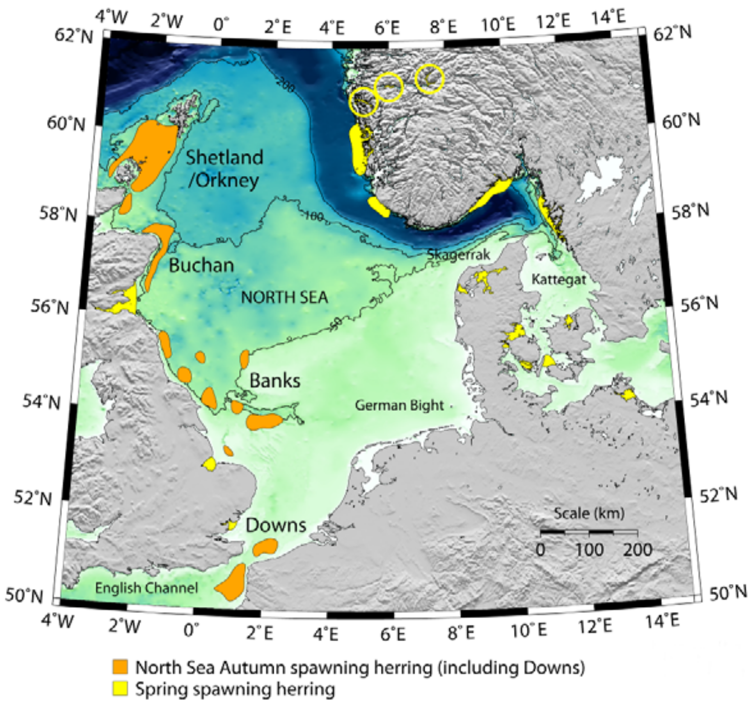

Figure 1.1: North Sea herring spawning components (from Dickey-Collas et al., in review)

To date, there is little discriminative power in using genetics to distinguish herring stocks in the NE Atlantic and spawner type (autumn, winter or spring) is not genetically determined (Gaggiotti *et al.*, 2009). Analysis of otolith microstructure can be used in the discrimination of mixed herring stocks (e.g. Clausen *et al.*, 2007; Bierman *et al.*, submitted). However, this is a time-consuming method during which each otolith needs to be polished in order to view the microstructure at the core of the otolith. Otolith shape analysis (Burke *et al.*, 2008a; Burke *et al.*, 2008b) is a faster and easy alternative method to determine stock identity. Also, the otolith is maintained in a condition so that it may be used in future analysis (Campana and Casselman, 1993; Burke *et al.*, 2008).

The aim of this project was to develop a technique that is cost-effective, robust to scientific review and simple in terms of planning in which morphometric shape differences are used to distinguish Norwegian Sea spawning herring and North Sea herring from each other. This research will be the first phase in a larger project to estimate the abundance of North Sea herring in the catch in the Norwegian Sea.

2 Materials and Methods

2.1 Samples

Jaczon BV and Van der Zwan provided IMARES with unsorted herring samples (frozen blocks of 20-23 kg) that were caught by the Dutch pelagic trawlers SCH123 and SCH54 in the Norwegian Sea from August-November 2008. Jaczon BV provided 22 (SCH123) and Van der Zwan 36 samples (SCH54). Because both companies provided more samples than needed we randomly selected (using www.randomizer.org) 25 samples: 9 samples and 16 samples from Jaczon and Van der Zwan respectively (Figure 2.1, Appendix 1). The fishers also provided photos of individual fish, as initially this project proposed to use morphometrics of total fish shape as a way to distinguish between herring types. However, early on in the development stage, it was found that the technology was not yet advanced enough to use whole fish shape to determine herring type.

A sub-sample of 25 fish, that was representative for the length distribution of the entire sample, was taken from each sample. Several biological measurements were taken of each fish; length, weight, sex and maturity. In addition, a picture of every fish in which all major features such as fins, gills, eye, tail etc. were visible and an otolith sample was taken. The first 9 otolith samples were embedded in histokit so that the otoliths could be aged by following the standard ICES protocol. However, it appeared technically challenging to digitise otoliths as the light may be reflected in an undesirable way. We therefore decided to store the remaining 16 otolith samples completely 'loose' in plastic trays.

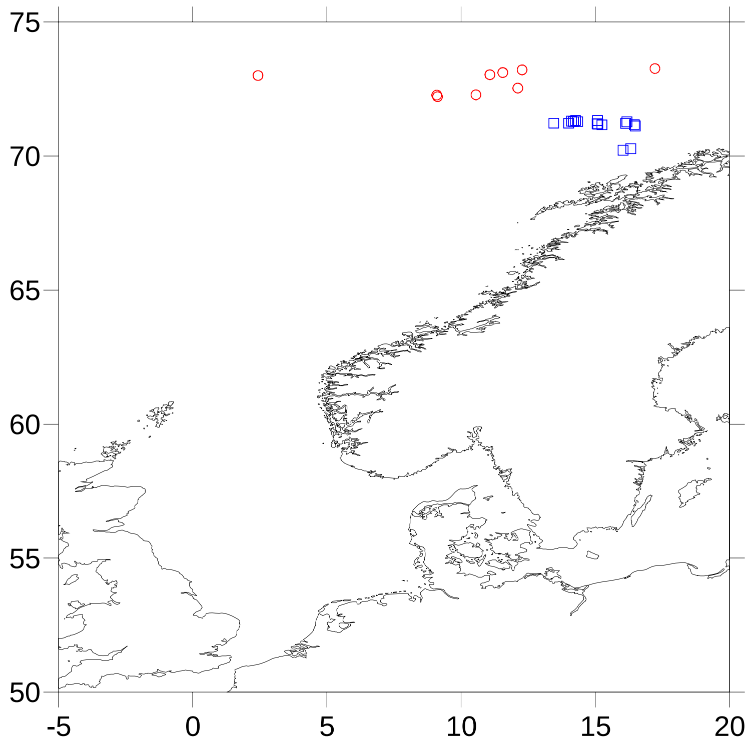

Figure 2.1: Positions of the 25 samples from SCH54 (blue squares) and SCH123 (red circles).

2.2 Determination of spawner type

DTU Aqua, Copenhagen, has developed a technique for morphometric discrimination of otoliths and fish. This technique can be used to determine the spawning origin of herring. In May 2009 a scientist from IMARES visited DTU Aqua to learn the technique for morphometric discrimination. The technique for morphometric shape discrimination of whole total fish was discussed during the visit in Copenhagen. It became clear that for this technique all photographs have to be analysed manually and was thus very labour intensive and relatively costly. This means that parameters such as length between gill and eye have to be manually measured for each fish. At present it is not possible to discriminate between spawning types with morphometric shape discrimination by applying a standard procedure. It was therefore decided not to continue with this procedure as it would be too time consuming.

2.2.1 Morphometric discrimination of otoliths

The technique for morphometric discrimination of otoliths involves taking a photograph of the complete otolith using Olympus Cell^D software. The otolith has to be placed on a black background, with the rostrum pointing to the right and the ventral edge pointing downwards (Figure 2.2; Appendix 2), under a dissecting microscope with a magnification of 20x10. Focus has to be set on the edge of the otolith and the contrast between the otolith and background should be made as clear as possible. There should be no shadows along the edge of the otolith and the reflection from the otolith and background has to be minimal. The image has to be calibrated, preferably before the otolith is photographed (Clausen, 2009). In general, we photographed the right otolith. However, when the right otolith was damaged, the left otolith was photographed and flipped around its horizontal axes. The Region Of Interest (ROI) of the otolith is outlined within the software programme ImageJ after which the image is saved as a black-and-white image (Figure 2.2). Thereafter an Elliptical Fourier Analysis (EFA), which fits a closed curve around the outer contour of the otolith, has to be run on the ROI with the software programme Shape. This results in a list of 40 sets of Fourier descriptors that together approximate the shape of the otolith. The more sets you use, the more detail is captured in the approximation.

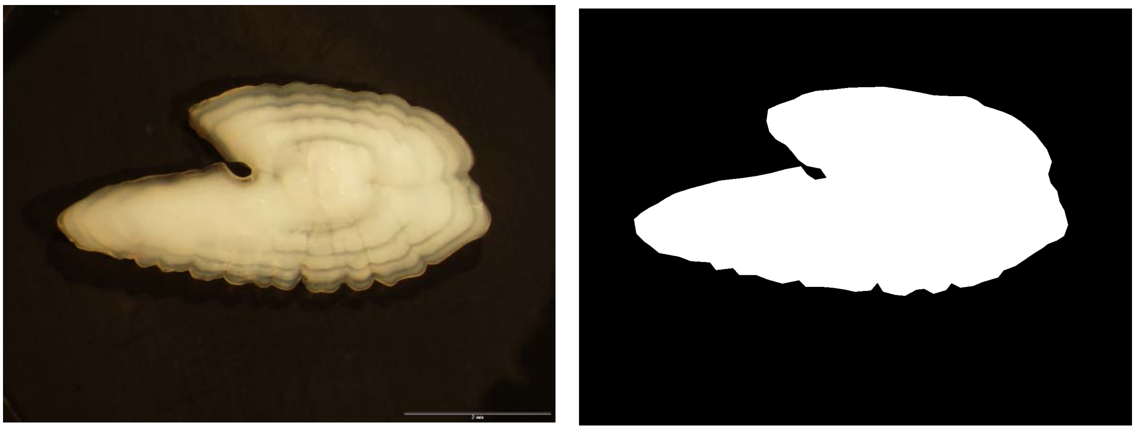

Figure 2.2: Photo of a herring otolith (left) and corresponding threshold image (right) that is used for Elliptical Fourier Analysis.

Otoliths

In order to determine the spawning types of the different otoliths, first a baseline had to be created. This baseline consisted of 31 definite Norwegian spring spawners, 25 definite North Sea winter spawners and 12 definite North Sea autumn spawners (Table 2.1; Figure 2.3). The otoliths originated from different samples and were all loose, i.e. not embedded. The spawning origin of the otoliths was either determined by using the pattern in the microstructure of the otolith core (Mosegaard *et al.*, 2001) or already known because they were caught on their spawning ground during the spawning season assuming that herring return to their spawning ground.

The technique for morphometric discrimination of otoliths was applied on the baseline samples. The Fourier descriptors that we obtained from this were used to set up a classification model. Thereafter, the morphometric discrimination technique was applied on the remaining 15 loose NORDIS otolith samples[1]. Finally, we embedded

[1] One of the 16 samples was used for the baseline

two of the 15 samples after which the technique was run again. This enabled us to determine whether the embedding process affects the outcome of the morphometric discrimination analysis. Because the light was reflected in an undesirable way when photographing embedded otoliths, oil had to be placed on top of the otolith in order to flatten the surface and a ring light had to be used when taking photographs.

Table 2.1: Overview baseline otoliths: number of samples (K), number of otoliths (N), determination of spawning origin and source of samples.

	K	N	Determination spawning origin	Source
Norwegian spring spawners	1	22	Microstructure analysis	NORDIS sample
Norwegian spring spawners	3	9	Targeted spawning ground	Provided by A. Geffen[2]
North Sea winter spawners	13	25	Microstructure analysis	(Bierman *et al.*, Submitted)
North Sea autumn spawners	9	12	Microstructure analysis	(Bierman *et al.*, Submitted)

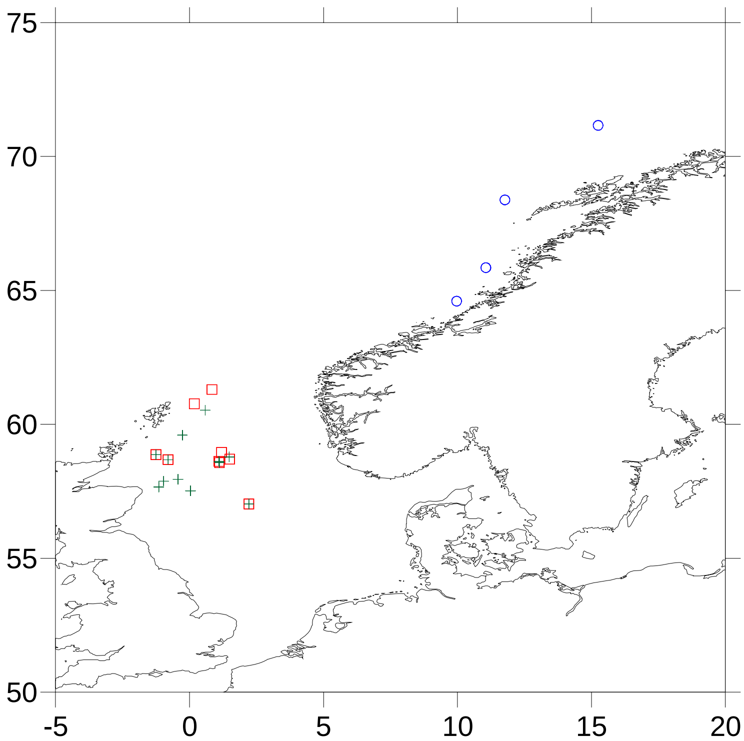

Figure 2.3: Positions of the baseline samples; North Sea winter spawners (green crosses), North Sea autumn spawners (red squares) both caught in the summer fishery and determined by otolith microstructure and Norwegian spring spawners (blue circle).

2.2.2 Classification model

The outline of each otolith is defined by a list of 40 Fourier descriptors each consisting of 4 measurements resulting in a total of 160 measurements. In our analysis 157 measurements could be used. First, the parameters from the baseline samples were analysed. Various exploratory plots of the parameters were made to explore whether measurements are more similar within spawner types than between spawner types. The analysis shows that there are differences in profiles between Norwegian spring spawners and the North Sea autumn or winter spawners; there appears to be a difference in the ostium area of the otoliths (Figure 2.4; Appendix 2 and 3).

[2] Audrey Geffen, University of Bergen, Department of Biology

Subsequently a Principal Component Analysis (PCA) was run to determine which of the parameters explain the largest amount of variation in the dataset. It appeared that a combination of all parameters in 5 axes describe most of the variation (>90%) between the spawning groups. These axes were put into a Linear Discriminant Analysis (LDA) to determine which combination of these axes could be used to create a maximum difference between the spawning types and a minimum difference within each spawning type. This resulted into a classification model consisting of two linear discriminants. This model is able to distinguish Norwegian spring spawning herring from North Sea autumn and winter spawners. At present, it has not been possible to distinguish between North Sea autumn and winter spawners. The classification model was used to classify the baseline and NORDIS samples into the two different groups.

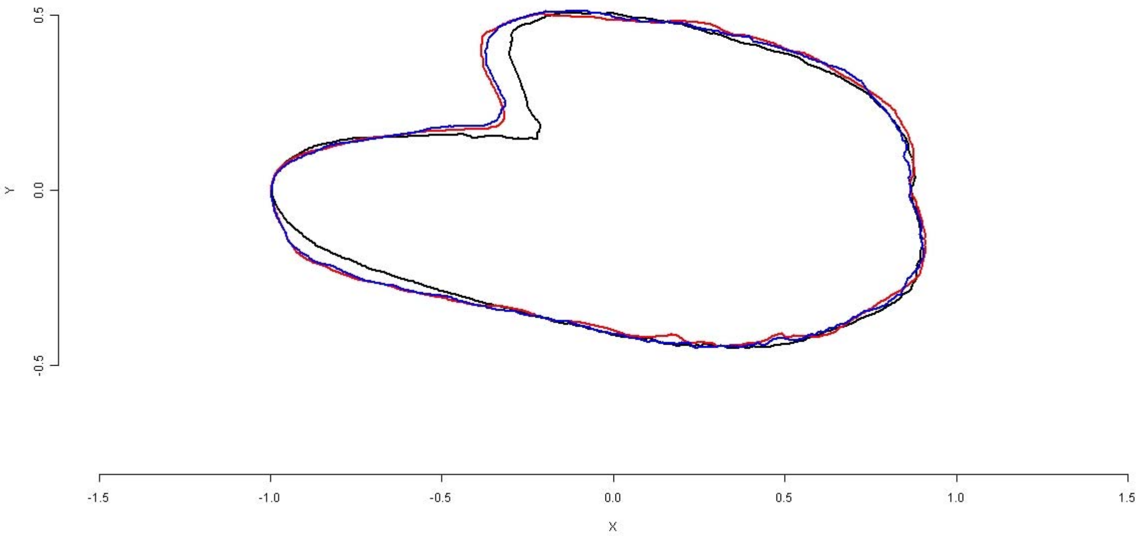

Figure 2.4: Median shapes of the baseline otoliths. Norwegian spring spawners (black line; based on 31 otoliths), North Sea autumn spawners (red line; based on 12 otoliths) and North Sea winter spawners (blue line; based on 25 otoliths).

3 Results

3.1 Baseline

The baseline samples were run through the model to determine its classification success. As expected from the exploratory analysis, there was a high classification success for Norwegian spring spawners; 28 out of 31 were classified correctly (Table 3.1). North Sea autumn and winter spawners could not be distinguished from each other and are therefore from now on referred to as the North Sea autumn or winter spawning group (see also Figure 3.1).

Table 3.1: Classification success of the model

		Classification model		
		Spring	Autumn	Winter
	Spring	28	2	1
Truth	Autumn	0	8	4
	Winter	1	9	15

3.2 Samples

The Fourier descriptors of the remaining 15 NORDIS samples were run through the classification model. The Fourier descriptors of the sixteenth sample were already run through as this sample was part of the baseline (Sample 10). The results show that based on our classification model 80% of the NORDIS samples are classified as Norwegian spring spawning fish and 20% as North Sea autumn or winter spawning fish (Table 3.2).

The graphical representation of the results also illustrates that a number of samples are not distributed in a similar manner as the Norwegian spring spawning group (Figure 3.1). In addition however, a few samples appear to cover an area that is not covered by the baseline study at all. Even so, it can be concluded that based on our classification model these otoliths still have a distinctly different shape than the otoliths originating from Norwegian spring spawning herring. At present these samples are classified as either Norwegian spring spawners or North Sea autumn or winter spawners. However, it is possible that these samples come from a herring type that we have not included in the baseline.

Table 3.2: Results from the classification model for the NORDIS samples

Sample	Spring	Autumn/ Winter
10	20	2
11		
12	18	5
13	19	5
14	18	6
15	20	5
16	20	3
17	20	3
18	17	8
19	21	4
20	19	3
21	21	4
22	17	6
23	20	5
24	19	4
25	18	6
Total	**267**	**67**
	80%	**20%**

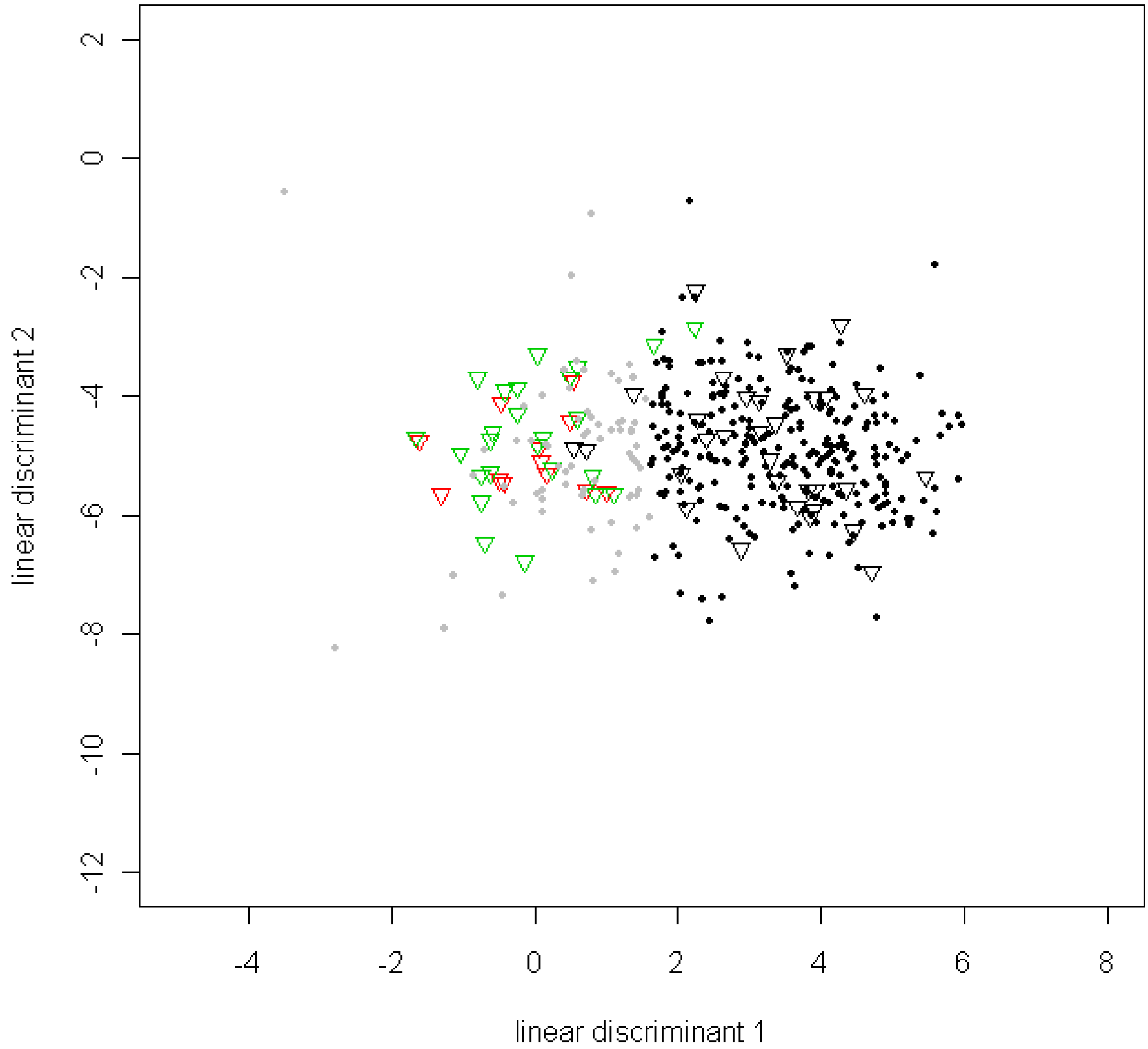

Figure 3.1: Graphical representation of the two linear discriminants that capture the maximum difference between spawning types and the minimum difference within each spawning type for all the otoliths: baseline study Norwegian spring spawners (black open triangles), baseline study North Sea autumn spawners (red open triangles), baseline study North Sea winter spawners (green open triangles), NORDIS samples classified as Norwegian spring spawners (black dots) and NORDIS samples classified as North Sea autumn or winter spawners (grey dots).

3.3 Embedded otoliths

The effect of the embedding process on the morphometric discrimination analysis of otoliths was tested for two samples. Some otoliths were excluded from this comparison because during embedding they broke or air bubbles arose that could not be avoided in the analysis. This resulted in a total of 35 otoliths that could be directly compared by running the Fourier descriptors of both the 'loose' and the embedded otoliths through the classification model. The results showed that 8 otoliths (=23%) were classified as a different spawning group after embedding.

4 Discussion

Based on the methods and results presented in this report it appears that we have developed a technique that can be used to discriminate between the spawning origin of herring samples with the use of otolith shape. The baseline samples show that the otolith shape of Norwegian spring spawning herring is distinctly different from the otolith shape from North Sea autumn and winter spawning herring. There appears to be an overlap in otolith shape of North Sea autumn and winter spawning herring. Nonetheless, the classification model is robust enough to distinguish Norwegian spring spawning herring from the other spawning components.

Based on the classification model the otolith shapes of the NORDIS samples appeared to differ from the expected distribution. The otolith shapes of the majority of the samples coincided with the baseline otoliths of Norwegian spring spawning herring. However, a number of otolith samples appeared to have distinctly different shapes from Norwegian spring spawning herring. We therefore classify these fish as North Sea autumn or winter spawners. Furthermore, the results show that a few of the NORDIS samples have not been covered by our baseline study. At present these samples are classified as either Norwegian spring spawning or North Sea or autumn spawning herring. However, these samples probably come from a herring type that we have not included in the baseline (west of Scotland, Norwegian coastal, Icelandic summer spawners etc).

This study also shows that the technique of morphometric shape discrimination can be applied to embedded otoliths. However, it appears that the embedding process may affect the outcome of the classification. At present the cause of this is still unclear. There may also be some photographic variation between images of the same otolith.

Even though we still have to overcome some methodological problems we are confident that this research constitutes a first step towards developing a technique to monitor and originate catches of herring from the Norwegian Sea. We can conclude from the results presented in this report that we can make a distinction between spawning types on the basis of otolith shape. However, we do need more insight. We therefore strongly recommend that NORDIS be continued. In a follow-up we should further expand the baseline study and work up in a statistically robust manner more samples caught in the Norwegian Sea (provided by the PFA and taken during the Market Sampling Programme) to begin to determine the origins of the herring catch in this area.

Acknowledgements

We would like to thank Lotte Clausen, Agnethe Hedegaard, Henrik Mosegaard and Jette Sandsted (all from DTU Aqua) for teaching us the technique for the morphometric discrimination of otoliths. We would further like to thank Audrey Geffen (University of Bergen) for providing us with Norwegian spring spawning samples and for her invaluable input in developing and writing up a standard protocol for otolith shape analysis. Finally, we would like to thank Niels Hintzen for his comments on the report.

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Quality Assurance

IMARES utilises an ISO 9001:2000 certified quality management system (certificate number: 08602-2004-AQ-ROT-RvA). This certificate is valid until 15 December 2009. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Environmental Division has NEN-AND-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2013 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

Justification

Rapport C142/09
Project Number: 430.11041.01

The scientific quality of this report has been peer reviewed by the a colleague scientist and the head of the department of IMARES.

Approved: N.T. Hintzen
Research scientist

Signature:

Date: December 21, 2009

Approved: J. Asjes, MSc.
Head of Department

Signature:

Date: December 21, 2009

Number of copies:	10
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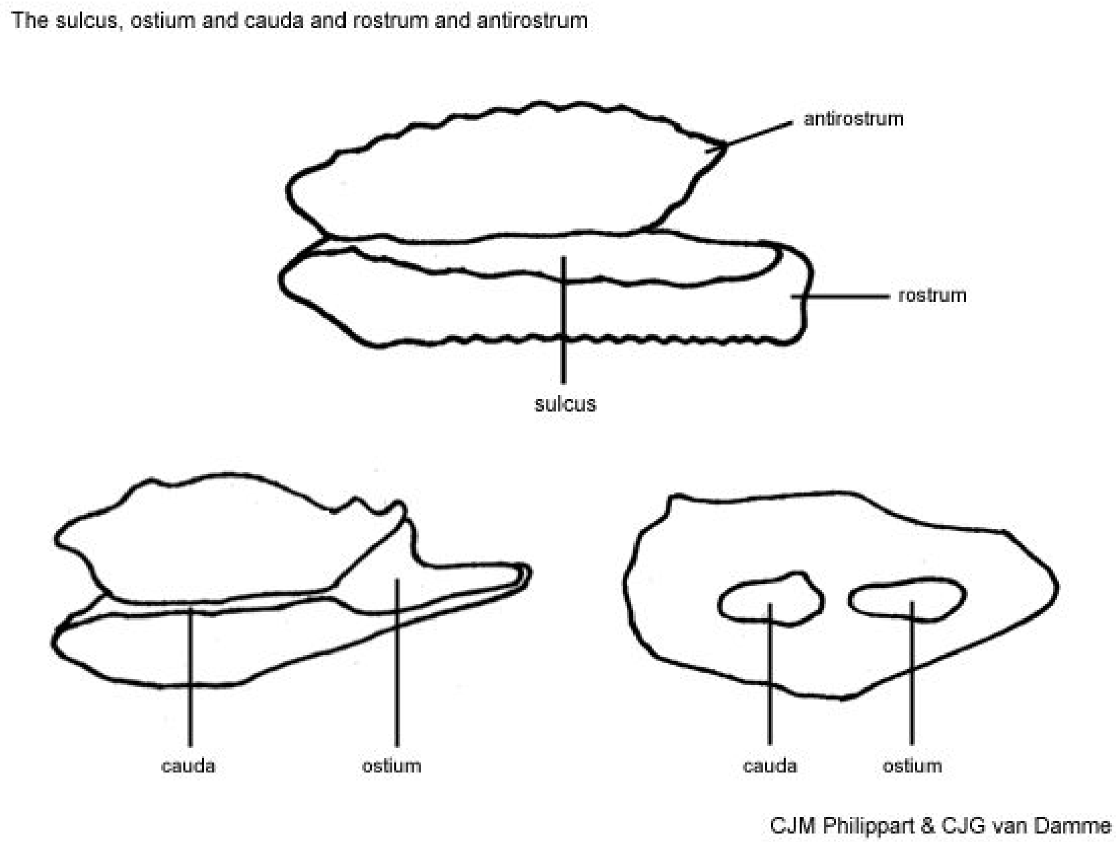
Number of graphs:	10
Number of appendix attachments:	3

Appendix 1

Overview of the 25 samples used for this research

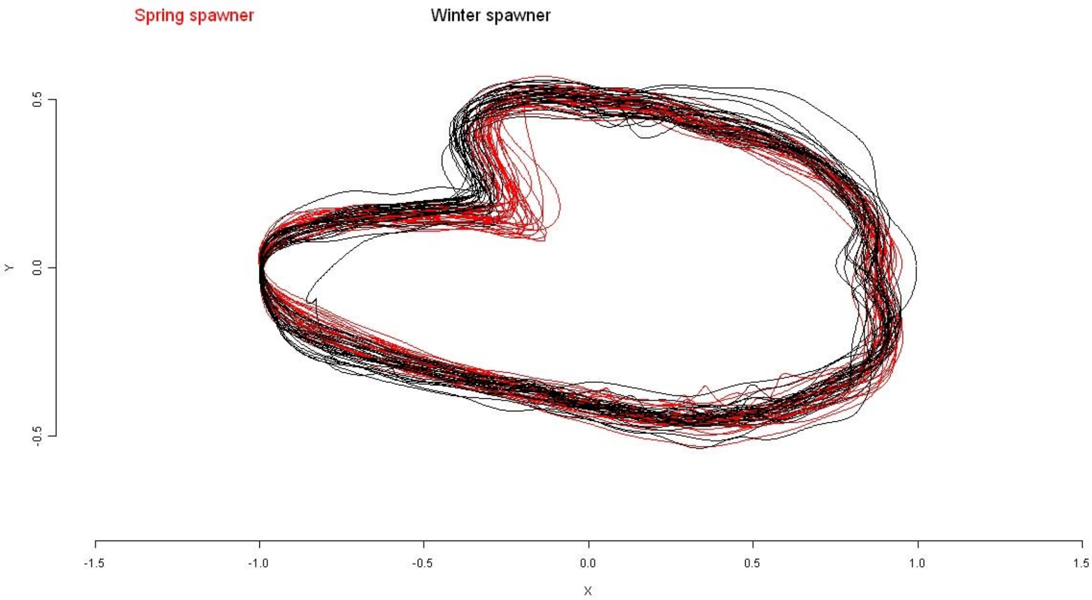
Ship	Sample	Date	Position
SCH123	1	03-09-2008	73°11’ N – 11°55’ E
	2	26-08-2008	72°21’ N – 09°12’ E
	3	04-09-2008	73°03’ N – 11°07’ E
	4	30-08-2008	72°53’ N – 12°11’ E
	5	01-09-2008	73°00’ N – 02°43’ E
	6	02-09-2008	73°21’ N – 12°27’ E
	7	03-09-2008	73°26 N – 17°22’ E
	8	07-09-2008	72°28’ N – 10°55’ E
	9	09-09-2008	72°27’ N – 09°09’ E
SCH54	10	18-10-2008	71°16’ N – 15°25’ E
	11	20-10-2008	70°21’ N – 16°03’ E
	12	20-10-2008	70°27’ N – 16°32’ E
	13	20-10-2008	71°27’ N – 16°18’ E
	14	23-10-2008	71°12’ N – 16°49’ E
	15	23-10-2008	71°17’ N – 16°45’ E
	16	23-10-2008	71°22’ N – 16°13’ E
	17	24-10-2008	71°19’ N – 15°09’ E
	18	24-10-2008	71°20’ N – 15°07’ E
	19	25-10-2008	71°32’ N – 15°08’ E
	20	26-10-2008	71°32’ N – 14°26’ E
	21	26-10-2008	71°28’ N – 14°18’ E
	22	29-10-2008	71°30’ N – 14°11’ E
	23	02-11-2008	71°28’ N – 14°34’ E
	24	03-11-2008	71°22’ N – 14°00’ E
	25	04-11-2008	71°22’ N – 13°45’ E

Appendix 2

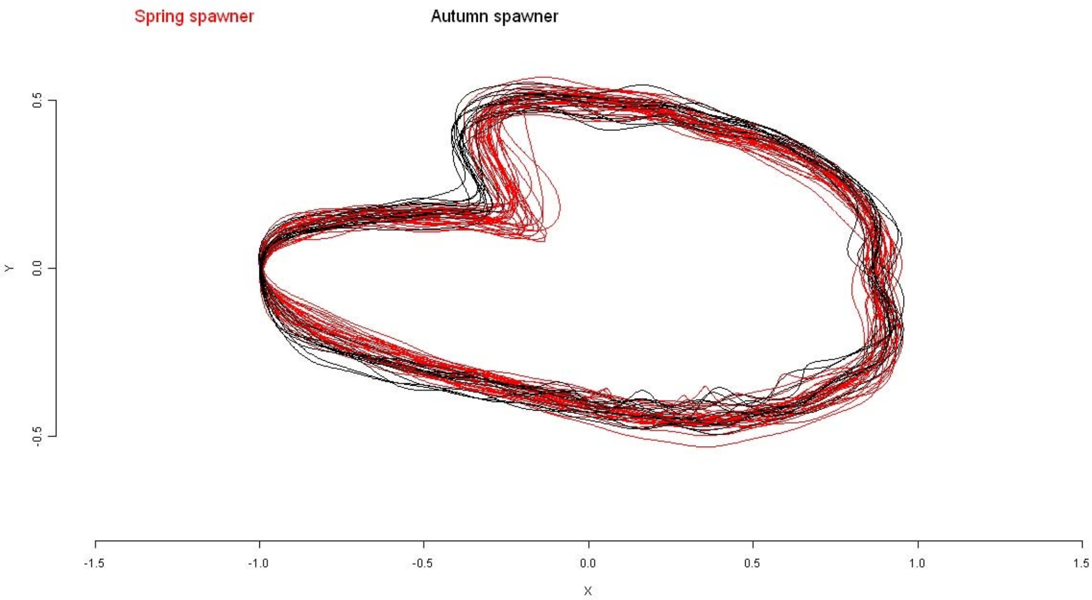


Schematic view otolith of the sulcus, ostium and cauda and rostrum and antirostrum (Leopold *et al.*, 2001)

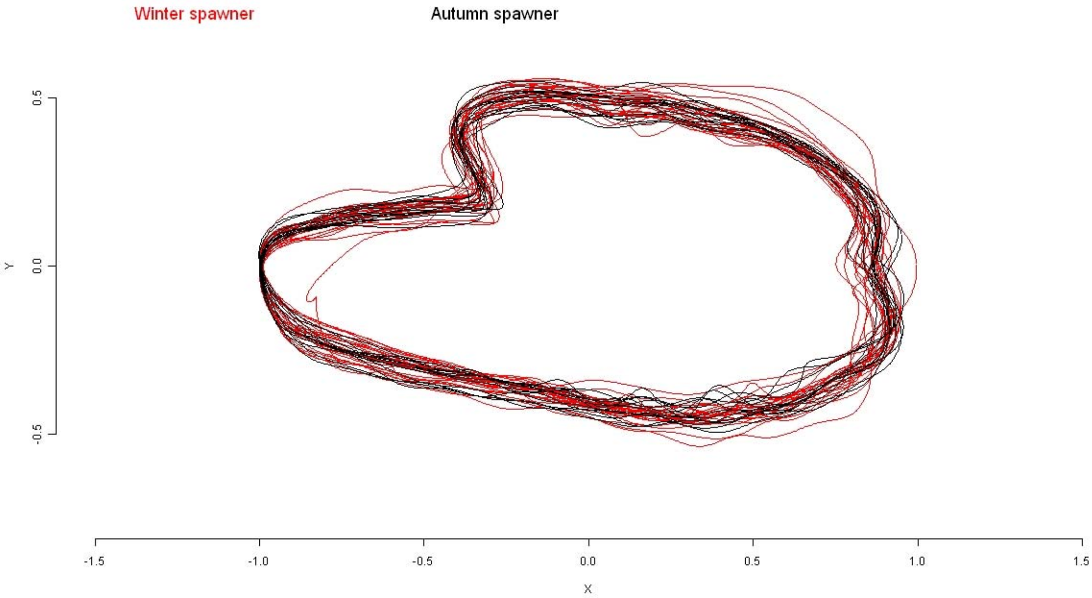
Appendix 3



Comparison of the shapes of the otoliths of the baseline Norwegian spring (red) and North Sea winter spawners (black)



Comparison of the shapes of the otoliths of the baseline Norwegian spring (red) and North Sea autumn spawners (black)



Comparison of the shapes of the otoliths of the baseline North Sea winter (red) and North Sea autumn spawners (black)